PROTECTIVE EFFECT OF PCI-NEO VECTOR-MEDIATED DNA VACCINE AGAINST CHLAMYDOPHILA ABORTUS IN MICE

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Received for publication February 25, 2008

Abstract

In the presented study, omp-1 gene coding main outer membrane protein of Chlamydia abortus was cloned into pCI-neo and pcDNA3.1 as delivery vehicles for DNA vaccination. Thirty-six BALB/c mice were randomly assigned to three groups and inoculated intramuscularly with: 1) 100 µg of pCI-neo, 2) 100 µg of pCI-neo::MOMP, and 3) 100 µg of pcDNA3.1::MOMP. All animals were vaccinated three times at 14 d intervals. The results showed that mice given pCI-neo::MOMP developed a higher IgG antibody level, high T lymphocytes proliferations, and high titres of IFN-γ and IL-2, than mice given pcDNA3.1::MOMP, which induced moderate antibody levels, less T lymphocyte proliferations and lower cytokine levels. No significant difference of TNF-α was observed in above groups. Additionally, IgG2a and Ig2b were the predominant isotypes on day 44, suggesting a high level of Th1 stimulation. Mice given the pCI-neo::MOMP also elicited a higher chlamydial clearance and a better protection than mice with pcDNA3.1::MOMP did. Immunisation with pCI-neo::MOMP vaccine may provide novel ways for active immunisation strategy against Chlamydia abortus.

Key words: mice, Chlamydia abortus, DNA vaccination, pCI-neo vector, pcDNA3.1 vector.

Chlamydia abortus (C. abortus) is an obligate intracellular Gram-negative bacterial pathogen, which colonises the epithelial cells of the reproductive tract and is responsible for abortion in pigs, cattle, and sheep. This pathogen becomes one of the most common causes of considerable loss in pregnant animals (5, 7, 9, 14, 17) Furthermore, it presents a zoonotic risk to pregnant women, since several cases of human chlamydial abortion have been reported (5, 6, 9).

The 40 kDa major outer-membrane protein (MOMP) is the predominant component and immunodominant antigen on the surface membrane of Chlamydiaceae, and plays a major role in the Chlamydiaceae infection (5, 8, 13, 14, 19, 21). MOMP harbours genus-, species-, and serotype-specific epitopes that elicit T-cell responses and neutralising antibodies, so it is considered to be the most likely vaccinal candidate antigen against C. abortus infections (13, 17). DNA vaccination has also been explored as a means of inducing protective immunity in a variety of animal model systems (5, 6, 13, 22). Recent studies have suggested that the combination of DNA and protein-based vaccines may be a more promising approach (23, 25). However, none of these approaches have so far generated immune responses, which are comparable to those produced by the current 1B attenuated vaccines (14).

For the current study, we have cloned the omp-1 gene into pcDNA 3.1 vector and pCI-neo vector then used both vectors as delivery vehicles for DNA vaccination.

Material and Methods

Bacterial strain. C. abortus CP 12 strain used in the study was originally isolated from an aborted piglet and supplied by the Institute of Microbiology and Epidemiology at the Military Medical Sciences Academy (Beijing, China). The bacteria were propagated in the yolk sac of embryonated SPF chicken eggs inoculated at day 7, and purified as previously described and stored at -80 °C (9, 13).

DNA vaccine construction. Genomic DNA as a PCR template was purified from C. abortus. The primers were designed based on sequence of the omp-1 gene from C. abortus (accession # AJ440239). The forward primer 5'-ATG AAA AAA CTC TTG AAA TCG G-3' and the reverse primer 5'-TTA GAA TCT
Antibody isotypes were determined using the r-MOMP as an antigen, as described previously (13, 14). Sections with 0.6% H2O2 in methanol and normal mouse staining were blocked by sequentially treating the endogenous peroxidase activity and non-specific paraffin, and sectioned at 5µm. After dewaxing, fixed in 4% formaldehyde solution, embedded in paraffin, then purified as described previously (13, 14).

**Immunisation and challenge.** All the experiments were performed on six-week-old female BALB/c mice (Beijing Vital Laboratory Animal Technology Company, Ltd.), which were maintained under pathogen-free conditions and treated in accordance with the guidelines issued by the Beijing Laboratory Animal Administration Committee on Animal Care. Thirty-six mice weighing 18-22 g were randomly divided into three equal groups and immunised on days 1, 14, and 28. Group 1 was immunised intramuscularly with 100 µg of pCI-neo vector, group 2 was inoculated with 100 µg of pcDNA3.1::MOMP, and group 3 was immunised with 100 µg of pCI-neo::MOMP. The mice were challenged on day 42 by intraperitoneal injection of 4 x 10⁵ plaque forming units (p.f.u.) of C. abortus CP12.

**Antibody responses.** Blood samples were collected on days 0, 14, 28, 42, and 49. Antibodies were detected with an indirect ELISA using C. abortus r-MOMP as an antigen, as described previously (13, 14). Antibody isotypes were determined using the antigen-mediate indirect ELISA. The isotypes were revealed by the addition of 100 µl of a 1/1000 dilution of peroxidase-conjugated horse anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA (Sigma). The OD was read at 450 nm after incubation with the substrate. Percentage of each IgG isotype was given as the ratio of the OD of each IgG subclass/total OD of IgG1, IgG2a, IgG2b, and IgG3 (14).

**Lymphocyte proliferations.** Six mice in each group were sacrificed and the spleens were collected on days 42 and 49. Single-cell suspensions of enriched T cells were prepared and the stimulation index (SI) was calculated as mean of the readings for antigen stimulated wells divided by mean for medium control wells, as described previously (13).

**Cytokine detection.** The levels of IFN-γ, TNF-α, and IL-2 were measured using mIFN-γ, mTNF-α, and mL-2 ELISA kit (Peprotech) following the manufacturer’s procedure. The culture supernatants of spleen cells were collected for the detection of the levels of IFN-γ, TNF-α, and IL-2 using mIFN-γ, mTNF-α, and mL-2 ELISA kit (Peprotech) following the manufacturer’s procedure. The MOMP antibody responses were shown in Fig. 1. As seen from the figure, mice immunised with pCI::MOMP induced significantly higher IgG antibody levels than mice given pcDNA3.1::MOMP on days 28 (P<0.05), 42 (P<0.01), and 49 (P<0.05). Although IgM antibody levels in mice immunised with pCI::MOMP or pcDNA3.1::MOMP were significantly higher than those of mice immunised with pCI-neo, no significant difference was found between pcDNA3.1::MOMP and pCI::MOMP approach (Fig. 2).

**Results**

**Antibody response.** The MOMP antibody levels were shown in Fig 1. As seen from the figure, mice immunised with pCI::MOMP induced significantly higher IgG antibody levels than mice given pcDNA3.1::MOMP on days 28 (P<0.05), 42 (P<0.01), and 49 (P<0.05). Although IgM antibody levels in mice immunised with pCI::MOMP or pcDNA3.1::MOMP were significantly higher than those of mice immunised with pCI-neo, no significant difference was found between pcDNA3.1::MOMP and pCI::MOMP approach (Fig. 2).

MOMP-specific IgG1, IgG2a, and IgG2b antibodies were found in the pool of sera of mice immunised with pCI::MOMP on days 28, 42, and 49. IgG2a was the predominant isotype on day 28, while IgG2a and IgG2b were the predominant isotypes on day 42 (Fig. 3). A lower IgG3 antibody level was detected at same period. No significant difference was found for antibody isotypes among IgG1, IgG2a, and IgG2b on day 49.

**T cell response.** Significant T cell responses were observed in mice with pCI::MOMP and pcDNA::MOMP on day 42 (Fig. 4). The T cell proliferation in mice inoculated with pCI::MOMP was significantly higher than that of mice with pcDNA::MOMP (P<0.05). Mice immunised with pCI::MOMP produced a weak T cell response as indicated by the T cell proliferation assay.

**Cytokines detection.** The levels of IFN-γ, IL-2, and TNF-α in the supernatant of splenocytes for all groups were shown in Fig. 5. Following three-fold immunisation, both IFN-γ and IL-2 levels were significantly higher in mice with pCI::MOMP than in mice with pcDNA::MOMP approach on days 42 (P<0.05) and 49 (P<0.05). However, no significant difference was observed for the levels of TNF-α in above groups. On day 49, IL-2 levels of two DNA vaccines were significantly lower than that of the vector group (P<0.05).

**Clearance of C. abortus in spleens.** The induced protection was evaluated by quantification of C. abortus in the spleen (Fig. 6). Chlamydial quantity in the spleen of mice with pCI::MOMP showed significantly higher clearance than that of the mice immunised with pcDNA::MOMP group (P<0.05).
**Fig. 1.** Kinetics of the IgG antibody response of the DNA immunised mice. 
**P<0.01** when compared with pcDNA3.1::MOMP. **P<0.05** when compared with pcDNA3.1::MOMP.

**Fig. 2.** MOMP-specific IgM antibody response of the mice immunised with DNA vaccine.  
* P<0.05 when compared with pCI-neo control group on days 42 and 49.

**Fig. 3.** Determination of anti-MOMP IgG subclasses in the sera of mice immunised with pCI::MOMP. 
The absorbance of each IgG subclass was divided by the value obtained by the addition of the absorbance of each IgG subclass.
**Fig. 4.** Proliferative responses of spleen lymphocytes from mice immunised on day 42 after inoculation. * P<0.05 when pCI::MOMP was compared with pcDNA3.1::MOMP group and when pcDNA3.1::MOMP was compared with pCI-neo vector group. ** P<0.01 when pCI::MOMP compared with pCI-neo vector group.

**Fig. 5.** Cytokine levels in the supernatant of splenocytes on days 42 and 49. The cytokines were tested individually by ELISA. * P<0.05 when compared with pcDNA3.1::MOMP group.
**Fig. 6. Chlamydia abortus** clearance in the spleen of the immunised mice. **P** < 0.01 when compared with pCI-neo vector group; *P* < 0.05 when compared with pCDNA3.1::MOMP group.

**Discussion**

The ultimate goal of chlamydial vaccine research is to identify protective vaccine delivery methods that are capable of inducing the necessary protective immune response. Central to this goal is the need to develop safe delivery vehicles and adjuvants for an optimal antigen presentation (5, 10, 12, 18). DNA vaccination, although promising in principle, has not been effective at protecting large animals from *C. abortus* (14, 17). It has been suggested that vaccine efficacy could be increased by modifying the basic DNA vaccination strategy, such as changing the delivery vehicle, the route of immunisation or using modified prime-boost vaccination strategies (17, 23). The reason for the lower efficacy of DNA vaccines in large animals and humans is unknown, but is likely to be a consequence of transfectional efficiency. Thus, improving the delivery of plasmid and the subsequent expression is critical for developing effective vaccines (1-3, 18). For this reason, we have tested two vectors as a delivery vehicle for a standard chlamydial DNA vaccine.

In this study, significantly higher antibody levels, higher T cells proliferation, as well as higher titres of cytokines were detected in the pCI::MOMP group as compared to the mice with pCDNA3.1-mediated vaccine. Similar to the pCI-neo vector, the pCDNA3.1 vectors contain the CMV enhancer/promoter region controlling the expression of the inserted DNA and the neomycin phosphotransferase gene under the regulation of the SV40 enhancer and early promoter. However, the expression of chloramphenicol acetyltransferase (CAT) gene in pCI-neo transfectants was 240 and 290-fold higher than that observed with pCDNA3-CAT in Hela cells (15). The low expression with the pCDNA3.1 vector is associated with the lack of an intron, which has been shown to enhance expression for many genes, and with the predicted presence of a large hairpin loop with a stability of -115 kcal/mol within the multiple cloning region (between the BamH I and Not I), which likely inhibits the translation (11, 16, 20). Antigen expression can be enhanced through the choice of promoter (e.g. human cytomegalovirus immediate early promoter) and by the insertion of a kozak sequence to optimise translational efficiency (16, 17). In the present study, pCI-neo vector with a β-globin/IgG chimerical intron located downstream from the enhancer/promoter region also facilitates DNA expression, leading to even higher levels of expression (11, 15). The increase of antigen expression resulted in the higher levels of serum IgG antibodies. The isotypes of anti-MOMP antibodies showed the pCI-neo::MOMP predominantly generated IgG2a antibody following the second immunisation, suggesting the major response was Th1-biased humoral immune response, which was consistent to the responses of the pCDNA3.1 ::MOMP vaccine (14, 17).

In the current study, IgM antibody level induced by pCI-neo::MOMP was significantly higher than that of pCDNA3.1::MOMP vaccine, suggesting that both two DNA vaccines are able to induce the memory immunity in mice. No serum IgA antibody was detected in the tested groups, suggesting the two vectors-mediated DNA vaccines did not induce mucosal immunity in mice. In the present study, pCI::MOMP vaccine induced high levels of cytokine IL-2 and IFN-γ in mice. A possible explanation for the higher level of T cell proliferation is that pCI::MOMP is taken up efficiently by antigen presenting cells of the hosts immune system, whereas pCDNA 3.1::MOMP vaccine will be taken up (less efficient) by a variety of cell types but most commonly muscle cells near the site of inoculation. Mice with pCI::MOMP vaccine also induced a higher level of T cell proliferations and higher chlamydial clearance in the spleen, which was clearly correlated to better protection (23, 26).

Mouse studies have shown that an MHC class, restricting CD4+ T helper type 1(Th1) response, mediated by IFN-γ and IL-2 production, is critical for resolving primary infection (5, 14, 26). Given the extreme importance of IFN-γ and IL-2 in host defence against *C. abortus* infection, these responses are particularly promising (5, 17, 21). In particular, the T
Acknowledgments: This work was supported in part by the Beijing Municipal Science and Technology Commission (No. 6052014) to Dr Cheng He.

References


